Alfalfa Seed Germination and Yield Ratio and Alfalfa Sprout Microbial Keeping Quality Following Irradiation of Seeds and Sprouts†

KATHLEEN T. RAJKOWSKI* AND DONALD W. THAYER

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 01-78: Received 1 March 2001/Accepted 2 July 2001

ABSTRACT

Foods can be treated with gamma radiation, a nonthermal food process, to inactivate foodborne pathogens and fungi, to kill insects on or in fruits and vegetables, and to increase shelf life. Gamma irradiation is especially well suited for these treatments because of its ability to penetrate commercial pallets of foods. Irradiated fruits, vegetables, poultry, and hamburger have been received favorably by the public and are now available in supermarkets. The use of irradiation on fresh alfalfa sprouts was studied to determine its effect on keeping quality as related to aerobic microbial load. After an irradiation dose of 2 kGy, the total aerobic count decreased from 10^{5-8} to 10^{3-5} CFU/g, and the total coliform counts decreased from 10^{5-8} to 10^{3-0} CFU/g. The results showed that the sprouts maintained their structure after irradiation, and the keeping quality was extended to 21 days, which is an increase of 10 days from the usual shelf life. The effect of various doses of irradiation on alfalfa seeds as measured by percent germination and yield ratio (wt/wt) of sprouts was determined. There was little effect on the percent germination, but as the dose increased, the yield ratio of alfalfa sprouts decreased. As the length of growing time increased, so did the yield ratio of the lower dose irradiated seeds (1 to 2 kGy). The irradiation process can be used to increase the shelf life of alfalfa sprouts, and irradiating alfalfa seeds at doses up to 2 kGy does not unacceptably decrease the yield ratio for production of alfalfa sprouts.

Over 300,000 tons of sprouts are produced annually for the U.S. market *(34)*. The seedlings or sprouts are grown hydroponically from a variety of seeds by commercial producers or by the consumer in the home. Sprouts are usually eaten raw in salads and can be cooked, as in Oriental-type meals. Of those varieties consumed raw, alfalfa sprouts are the most common. Twelve confirmed foodborne outbreaks occurred between 1995 and 1999 after eating pathogen-contaminated raw alfalfa sprouts *(27, 34)* caused by either *Escherichia coli* O157:H7 or *Salmonella* spp. The source of the pathogens was traced to contaminated seeds *(27)*. As a result of these outbreaks, the U.S. government has declared raw sprouts, particularly alfalfa sprouts, a high-risk food for the general public. The Food and Drug Administration (FDA) now recommends that, prior to sprouting, all seeds be disinfected by washing in a solution of 20,000 ppm Ca hypochlorite. This treatment can only reach pathogenic microorganisms on the seed surface, and there is still no guarantee that all contamination will be removed *(26)*. Due to the unreliability of the disinfectant step, the FDA is requiring the testing of spent irrigation water for the pathogens within 24 h after the start of the sprouting procedure. A more thorough history of sprout-related foodborne outbreaks and risk factors associated with the consumption of raw sprouts can be found elsewhere *(27, 34)*.

Various methods used to disinfect sprout seeds of the bacterial pathogen were reviewed in reports by the National Advisory Committee on Microbiological Criteria for Foods *(27)* and by Thompson and Powell *(34)*. Some of the unsuccessful sanitizers investigated for surface decontamination of the seeds are sodium or calcium hypochlorite, hydrogen peroxide, chlorine dioxide, ethanol, oxonated water, acidified sodium chlorite, organic acid/hypochlorite, and gaseous acetic acid *(12, 25, 27)*. These surface treatments cannot guarantee that the interior of the seeds is also decontaminated *(27)*. Low-dose irradiation can be successfully used as a seed fumigant against fungal growth. There are numerous reports of this treatment for a variety of seeds, i.e., soybean, cotton, peanut, maize, onion, barley, chickpea, Brazilian bean, and corn *(4, 5, 10, 11, 22, 30, 31, 37, 39)*. In addition to controlling the fungal contamination, the germination of the seeds was not affected by this low-dose irradiation treatment. Recently, the FDA gave final approval for the use of irradiation up to 8 kGy to control microbial pathogens in seeds for sprouts *(35)*. However, there are few or no reports on the microbial decontamination of alfalfa seeds using low-dose ionizing irradiation and the resulting effect of irradiation on alfalfa seed germination or yield ratio (wt/wt).

Several methods have been unsuccessful at decontami-

* Author for correspondence. Tel: 215-233-6440; Fax: 215-233-6559; E-mail: krajkowski@arserrc.gov.

† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

nating raw vegetables of bacterial populations, including pathogens. Bari et al. *(3)* showed that the introduction of calcinated calcium to the radish sprout growth medium either completely inhibited or inactivated *E. coli* O157:H7. Others have reported on the use of disinfectant solutions to remove pathogens from cut lettuce. Beuchat *(8)* reported that washing with water or 200 ppm of chlorine solution had little effect on the removal of *E. coli* O157:H7 from lettuce. Using a combination of 0.5% lactic acid plus 100 ppm of chlorine, Escudero et al. *(14)* reduced numbers of *Yersinia enterocolitica* by 6 logs. Since there is no way to guarantee pathogen-free raw sprouts, the FDA in 1999 issued a warning about the hazards of eating raw sprouts *(36)*.

Several studies have reported on the successful reduction and increased keeping quality of fresh-cut vegetables *(15, 16, 20, 21, 28)* using low doses of irradiation. A study by Rajkowski and Thayer *(29)* reported *D*-radiation values for *E. coli* O157:H7 and *Salmonella* spp. on raw sprouts. A 2-kGy dose of irradiation would achieve a 5-log kill of these pathogens on the sprouts. There are no reports as to the keeping quality of the sprouts after irradiation at 2 kGy.

The purpose of this study was to determine the effect on germination and yield of irradiated alfalfa seeds and the keeping quality of irradiated alfalfa sprouts.

MATERIALS AND METHODS

Irradiation of samples. All samples were irradiated using a self-contained gamma-radiation source of ^{137}Cs, with a strength of approximately 109,159 Ci (4.039 Pbq) and a dose rate of 0.10 kGy min^{-1}. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology, Gaithersburg, Md. The actual dose was verified by reading dosimeter alanine pellets on an electron paramagnetic resonance analyzer (EMS 104 EPR; Bruker, Rheinstellen, Germany). The sample temperature during irradiation was monitored continuously during irradiation and maintained by injecting the gas phase of liquid nitrogen into the irradiation chamber.

Seeds: temperature effect on irradiated seed yield ratio. Alfalfa seeds (Caudill Seed Co., Louisville, Ky.) were irradiated to 5 kGy at 0, 5, 10, 15, 20, 25, and 30°C. Duplicate 1-g samples at each temperature were placed in individual growth trays. Then, the samples were put in a tray sprouter with an automatic irrigation system that sprayed water for 1 min every 30 min. After 4 days of growth, the sprouts were weighed, and the yield ratio was calculated by dividing the sprout weight by the weight of seeds. The procedure was repeated.

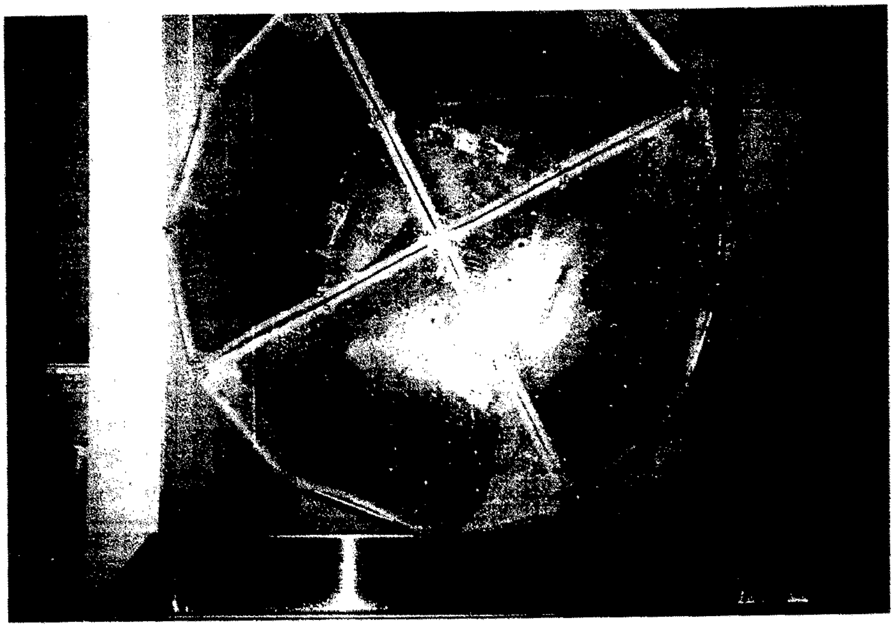
Seeds: radiation dosage effects on seed germination and yield ratio. After determining no temperature-irradiation effect on the yield ratio, all subsequent procedures were done at 20°C. Alfalfa seeds were irradiated to doses of 1, 2, 3, 4, and 5 kGy. The percent germination was determined by counting out 100 of the irradiated and nonirradiated seeds and germinating according to the method described by the Association of Official Seed Analysts *(1, 2)*. The percent germination was done in duplicate and repeated, whereas the yield ratio determination was done in quadruplicate and repeated. The seeds were stored at room temperature for 12 months, and the percent germination and yield ratio determination were repeated. The yield ratio of the irradiated seeds was determined by the procedure described above.

FIGURE 1. *Commercial tray sprouter.*

Seeds: yield ratio obtained by commercial producer. One hundred fifty-pound portions of alfalfa seeds (International Specialty Supplies, LLC, Cookeville, Tenn.) were irradiated at 20°C to 1, 2, and 3 kGy; given to a commercial sprouter (Snider's Sprouts, Potomac, Md.) to determine the yield ratio; and processed according to the FDA's recommendations *(27)*. The seeds were sanitized in a 20,000-ppm Ca hypochlorite solution (Olin Corp., Norwalk, Conn.), rinsed with tap water, placed in a rotating drum for 24 h, and removed; then, a known weight was placed in trays before being put in the growth cabinet (Figs. 1 and 2). The growth cabinet held 20 trays and was fitted with an automatic sprayer (1 min every 30 min). Five trays of seeds irradiated at 1, 2, and 3 kGy plus the control were placed in the growth cabinet. The sprouts were harvested 4 days later, and the yield ratio was obtained (weight of sprouts divided into weight of seeds). The top two and bottom two slots were not used.

Sprouts: sprout keeping quality preparation. Market-packed alfalfa sprouts were obtained from local growers and delivered within 24 h after harvesting. The sprouts were kept refrigerated (4 to 8°C) during transportation and storage. The sprouts were irradiated to a dose of 2 kGy at 20°C. The irradiated alfalfa sprouts were kept refrigerated (4 to 8°C) until used.

FIGURE 2. *Commercial drum sprouter.*



Sprouts: microbiological analysis. Immediately after irradiation and at weekly intervals, irradiated and control market packages of sprouts were examined visually and the sprouts photographed. The alfalfa sprouts were analyzed for total aerobic and coliform bacteria counts. After a 1/10 dilution with buffered peptone water (Difco Laboratories, Detroit, Mich.) and serial diluting with buffered peptone water, the total aerobic and coliform counts were obtained using aerobic and *E. coli*-coliform PetrifilmsJ (3M Microbiology Products, St. Paul, Minn.), respectively. All plates were incubated at 37 ± 1°C for 24 h before being hand counted.

Seeds: estimate of sensorial keeping quality. The subjective changes in appearance were followed, along with the microbial analysis as a function of the 2-kGy radiation treatment and storage. Appearance, discoloration, softness by touch, and odor were scored, with scores ranging from 5 for excellent to 1 for unmarketable.

RESULTS AND DISCUSSION

Effect of temperature on alfalfa seed yield ratio (wt/wt). The temperature during irradiation was varied from 0 to 30°C in 5°C increments to determine the effect on the yield ratio (wt/wt). The yield ratio is the amount of sprouts obtained after the proper growth time from a known amount of seeds. The sprout producers routinely test the purchased alfalfa seeds to verify an acceptable yield ratio of ≥10/1 (wt/wt). The 5-kGy dose was chosen so that a marked difference of ≥50% in growth between the control alfalfa sprouts and those raised from the irradiated seeds would be observed. The decrease in yield ratio (wt/wt) was 54% from a 9.9/1 (wt/wt) for the control versus 4.3/1 (wt/wt) for the 5-kGy treated alfalfa seeds. There was no difference in yield ratio due to temperature effect: average yield ratio for all temperatures studied was 4.3 ± 0.2/1 (wt/wt). All future irradiation procedures on the alfalfa seeds were done maintaining the temperature at 20°C.

TABLE 1. *% germination and yield ratio (wt/wt) of irradiated alfalfa seed stored 1 year*

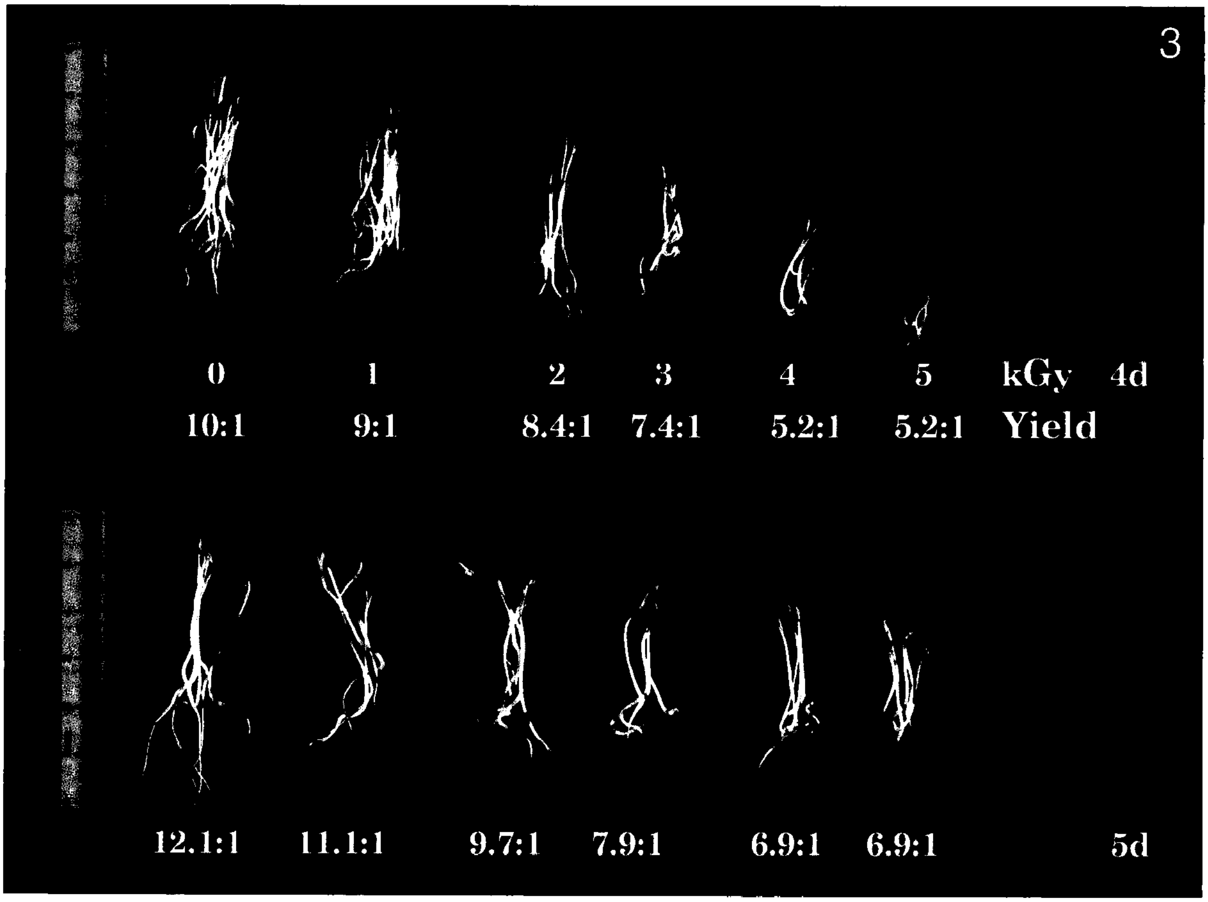
Irradiation dose (kGy)	% germination		Yield ratio (wt/wt) after:	
	After storage[a]	After storage[b]	4 days of growth	5 days of growth
0	88	80	10.1:1	12.1:1
1	98	36	9.1:1	11.1
2	99	28	8.4:1	9.7:1
3	97	28	7.4:1	7.9:1
4	98	27	5.2:1	6.9:1
5	98	24	5.2:1	6.9:1

[a] Seeds stored at room temperature for 6 months.
[b] Seeds stored at room temperature for 18 months.

Effect of irradiation dose on alfalfa seeds. The effect of varying dose rates from 1 to 5 kGy on alfalfa seeds' germination and yield ratio (wt/wt) was studied. Regardless of dose, the percent germination determined was initially the same for each dose level used (Table 1). The increase in percent germination of the irradiated seeds from the control is not unusual. Other researchers have noted that there was no effect or an increase in percent germination by treating seeds with radiation *(11, 19, 22, 30)*, while others have reported that if there was a decrease in percent germination, the restoration of viability occurred upon storage *(5)*. In this study, we observed marked decreases in the percent germination of the irradiated alfalfa seeds after 1 year of storage (Table 1).

There was a decrease in yield ratio (wt/wt) as the irradiation dose increased (Table 1) compared to no decrease in percent germination. Figure 3 illustrates this decrease in sprout size (less growth), which resulted in lower yield ratios as the dose rate increased. The yield ratio did increase when the growing time was extended by 24 h (5 days from start of growth) for the 1- and 2-kGy samples (Fig. 3), resulting in the ≥10/1 (wt/wt) level needed by the sprout producers.

Yield ratio obtained by commercial producer. To verify the laboratory results, alfalfa seeds were irradiated, labeled with a code, and given to the commercial sprout producer. All batches of seeds (control and 1-, 2-, and 3-



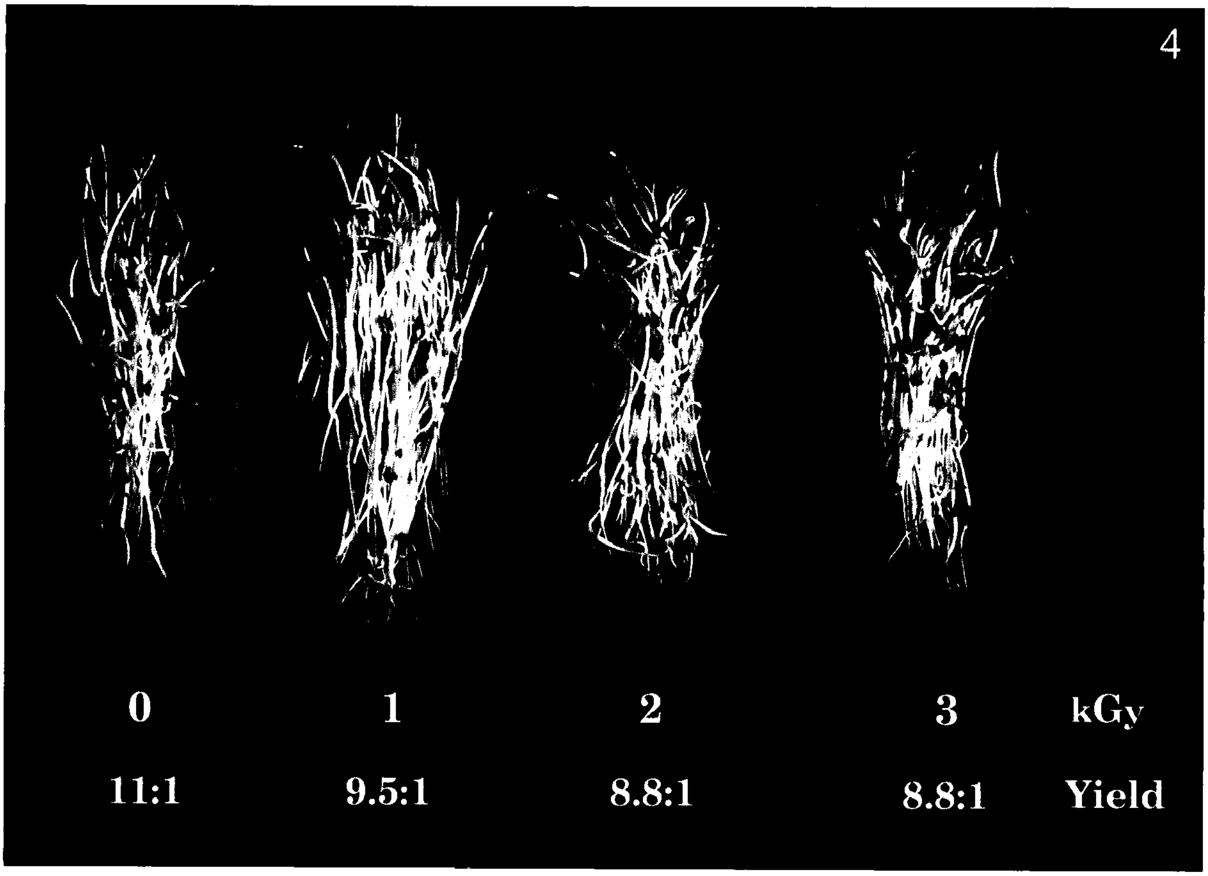


FIGURE 3. *Yield ratio (wt/wt) and length of alfalfa sprouts raised from irradiated seeds after 4 and 5 days of growth.*

FIGURE 4. *Yield ratio (wt/wt) of alfalfa sprouts raised at a commercial facility from irradiated seeds.*

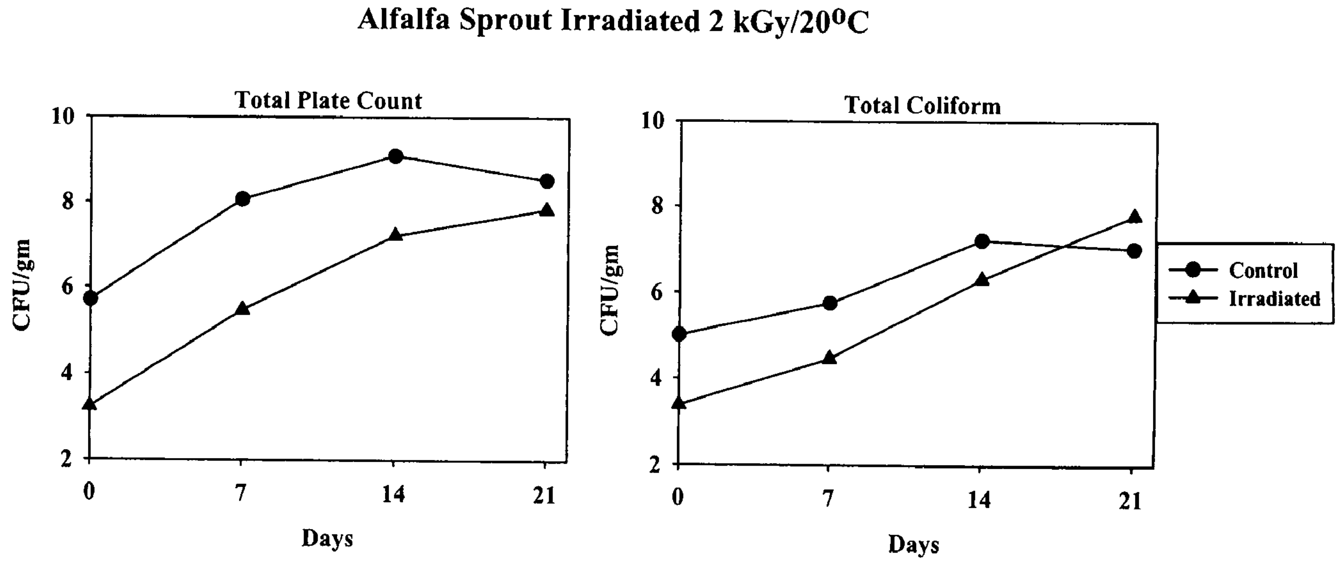


FIGURE 5. *Microbial change and regrowth of background microflora on alfalfa sprouts irradiated to 2 kGy.*

kGy irradiated alfalfa seeds) were processed according to FDA protocol and were placed in separate quadrants of the rotary drum after sanitizing and rinsing (Fig. 1). The next day, the wet seeds were removed from the drum. After weighing 2.05 kg of the wet seeds and placing in trays, they were placed in the growth cabinet (Fig. 2). Four days after the start of the procedure, the four trays of each sprout sample were weighed and averaged to obtain the yield ratio. Figure 4 illustrates a representative portion of sprouted seeds for each irradiation level. The yield ratio was 9.5/1, 8.8/1, and 8.8/1 (wt/wt) for the 1-, 2-, and 3-kGy irradiated alfalfa seeds, respectively. This represents a 14, 20, and 20% decrease in yield ratio for the 1-, 2-, and 3-kGy seeds, compared to the control, which had an 11/1 yield ratio. Even with this decrease, the yield ratio was accepted by the commercial sprout grower. The commercial sprout grower also did a yield ratio on totally drum-raised sprouts from the irradiated seeds. The yield ratios for the drum-raised seeds were 10/1, 9/1, 8/1, and 7/1 for the control, 1-, 2-, and 3-kGy seeds, respectively. These ratios compared well with the tray-raised sprouts, as this commercial grower usually only produces tray-raised sprouts.

Sprout keeping quality. The keeping quality of fresh alfalfa sprouts was determined. Sprouts, packaged in plastic "clamshell" containers, and unpacked sprouts (whole trays) were obtained from the supermarket and local sprout growers. Previously, it was reported that the aerobic background counts on fresh produce, including sprouts, could vary with counts from as low as $<10^2$ to as high as 10^7, depending on the produce, handling, and climate *(17, 18, 33)*. In this study, the ranges of total aerobic background counts on the fresh alfalfa sprouts were between 10^5 and 10^8 CFU/g before irradiating to 2 kGy and between 10^3 and 10^5 CFU/g after irradiation. The total coliform counts on sprouts before irradiation ranged from 10^5 to 10^8 CFU/g, and after irradiation, they ranged from 10^3 to 10^5 CFU/g. Regardless of when or where purchased, there was an average of a 2- to 3-log decrease in total aerobic counts and a 2- to 4-log reduction in total coliform counts.

Representative curves for the total aerobic and coliform counts are illustrated in Figure 5, which shows the increase in counts during storage. The total plate count increased, but not to the amount in the control, as did the total coliform count for the first 2 weeks.

Samples of the unirradiated and irradiated sprouts for each sampling time were photographed (Fig. 6) to show the deterioration upon storage. In addition to the photographic record, the color, appearance, feel, and odor were also recorded. The scores (1 to 5) of the control and irradiated alfalfa sprouts for the sensorial properties are listed in Table 2. By the second week of storage, the control samples began to show signs of deterioration by the change in color,

TABLE 2. *Effect of 2 kGy radiation on the keeping quality of alfalfa sprouts*

	Control					2 kGy				
Days of storage	Log CFU/g	Color	Appearance	Touch	Odor	Log CFU/g	Color	Appearance	Touch	Odor
0	5.7	5	5	5	5	3.2	5	5	5	5
7	8.1	5	4	5	5	5.5	5	5	5	5
14	9.1	3	3[a]	2[b]	4	7.2	5	5	5	5
21	8.5	1	1	0	0	7.8	4	4	4	4

[a] Value of 3 considered borderline acceptable.
[b] Value of 2 or below unacceptable.

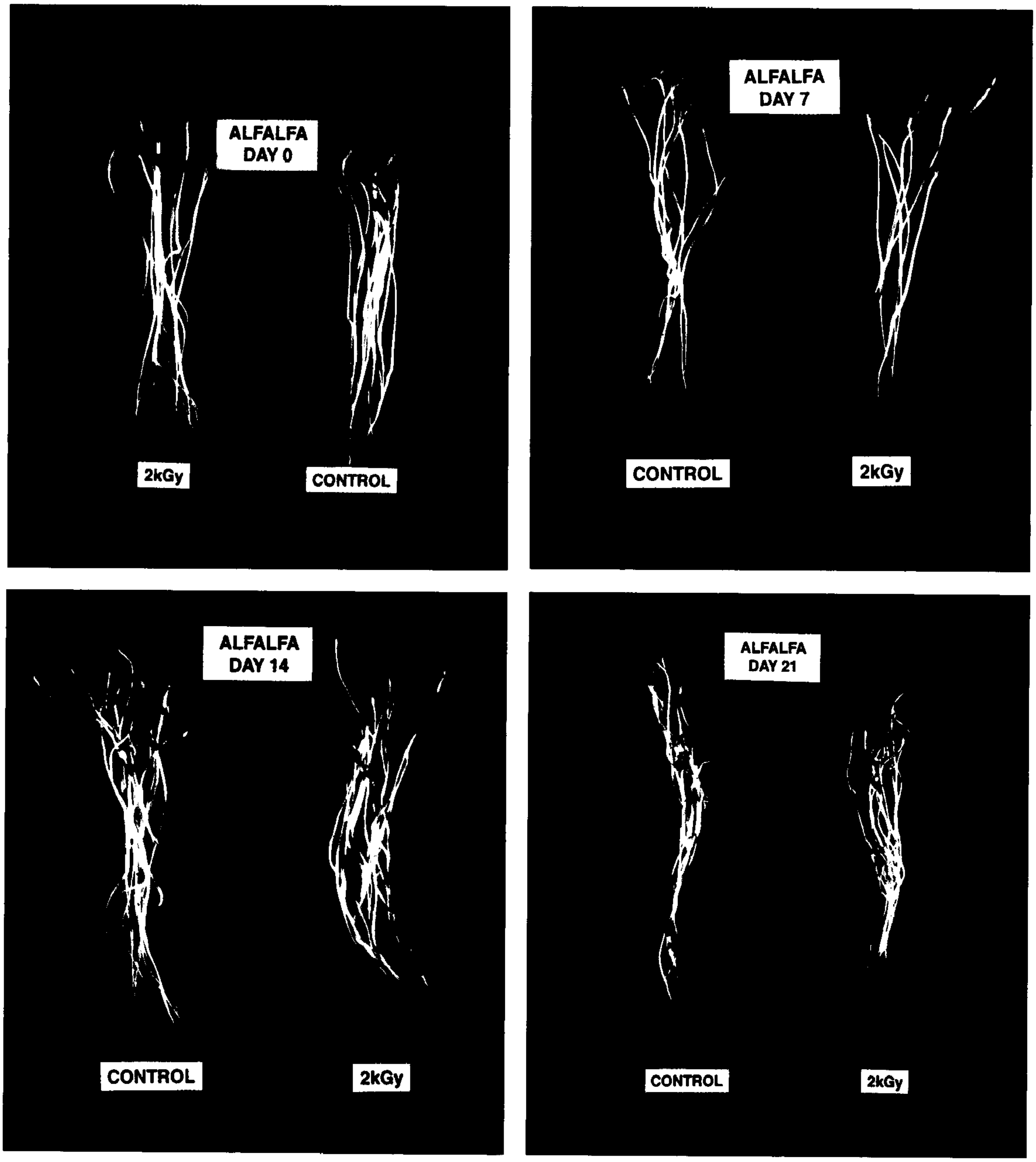


FIGURE 6. *Keeping quality of the irradiated alfalfa sprouts compared to the unirradiated.*

shriveled appearance, and slimy feel compared to the irradiated alfalfa sprouts. After 3 weeks of storage, the control samples had an off-odor, shriveled roots, and obvious browning and mycelia growth on the root. The shelf life of the alfalfa sprouts irradiated to 2 kGy was extended and, by the third week of storage, was just beginning to show signs of deterioration.

CONCLUSIONS

Seed decontamination is a twofold problem: the pathogen can be located under the seed coat or located in a crack or crevice where liquids may not penetrate *(9, 32, 38)*. When liquid disinfectants are in contact with the seeds for an extended time, there are reports of decreased germination *(9)*. Even with liquid disinfection of the seed, there is still no guarantee that the resulting sprout is pathogen free *(38)*. The FDA approved the use of irradiation up to 8 kGy to control bacterial pathogens on the alfalfa seeds used for sprouting. This study found that, as the dose increased above 3 kGy, the percent germination was not affected, but the yield ratio was decreased. Even extending the growth time by 24 h did not improve the yield ratio for those seeds irradiated at >3 kGy, which would not be acceptable to a sprout producer. Further studies are needed to determine the dosimetry of alfalfa seeds and the actual irradiation destruction value for microbial pathogens on dry alfalfa seeds needed to obtain a 5-log kill as required by the FDA. The combination of a liquid disinfectant or other agent and irradiation may provide for a risk-free sprout. Studies are also needed to determine if competitive exclusion can be useful. The natural alfalfa seed extract, canavanine, was shown to be effective in controlling the population of *Bacillus cereus* on the seeds *(13)*. Further studies are needed in this area to determine if the addition of the natural inhibitor to the water used in the sprouter can control other bacterial pathogens.

Surface disinfecting and packaging of produce under modified atmosphere packaging were reported to control the microflora on fresh fruits and vegetables *(6, 7, 23, 24)*. In these studies, the produce was not as fragile as sprouts. Rajkowski and Thayer *(29)* did show that a 5-log reduction of both *Salmonella* spp. and *E. coli* O157:H7 was obtained when the sprouts were irradiated to 2 kGy. In this study, we found that the keeping quality of the sprout was extended >10 days after irradiation at 2 kGy, the amount found for a 5-log reduction of the bacterial pathogens. Further studies on alfalfa sprouts are needed to determine if there are any nutrient or flavor changes in the irradiated sprout, and a trained sensorial panel should be used to rank the acceptability of the irradiated sprouts.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help and assistance of Nancy Snider of Snider Sprouts in performing the commercial verification of the yield ratio tests and Bob Rust and Dan Caudill for their contributions. Pablo Flores and June Leon did the technical work at the commercial operation, and Gina Bates and Dana Drazul did the laboratory technical work.

REFERENCES

1. Association of Official Seed Analysts. 1998. Germination tests. Rules for testing seeds. 18. Association of Official Seed Analysts, Lincoln, Nebr.
2. Association of Official Seed Analysts. 1992. Seedling evaluation handbook. Association of Official Seed Analysts, Lincoln, Nebr.
3. Bari, M. L., H. Kusunoki, H. Furukawa, K. Ikeda, K. Isshiki, and T. Umerura. 1999. Inhibition of growth of *Escherichia coli* O157:H7 in fresh radish (*Raphanus sativus* L.) sprout production by calcinated calcium. J. Food Prot. 62:128–132.
4. Basyony, A. E., M. I. El-Refaei, M. S. Galal, and M. I. E. Barakat. 1989. Effect of gamma irradiation on seed-borne fungi and soybean seed components during storage. Agric. Res. Rev. 67:619–628.
5. Berezina, N. M., A. A. Narimanov, A. F. Revin, and N. Norbeav. 1967. Restoration of radiation damages to cotton seeds during prolonged storage. UDC 58:140–145.
6. Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. 52:702–705.
7. Beuchat, L. R. 1992. Surface disinfection of raw produce. Dairy Food Environ. Sanit. 12:6–9.
8. Beuchat, L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. J. Food Prot. 62:845–849.
9. Caetano-Anollés, G., G. Favelukes, and W. D. Ba... 1990. Optimization of surface sterilization for legume seed. Crop Sci. 30:708–712.
10. Chiou, R. Y.-Y., C. M. Lin, and S.-L. Shyu. 1990. Property characterization of peanut kernels subjected to gamma irradiation and its effect on the outgrowth and aflatoxin production by *Aspergillus parasiticus*. J. Food Sci. 55:210–217.
11. Cuero, R. G., J. E. Smith, and J. Lacey. 1986. The influence of gamma irradiation and sodium hypochlorite sterilization on maize seed microflora and germination. Food Microbiol. 3:107–113.
12. Delaquis, P. J., P. L. Sholberg, and K. Stanich. 1999. Disinfection of mung bean seed with gaseous acetic acid. J. Food Prot. 62:953–957.
13. Emmert, E. A. B., J. L. Milner, J. C. Lee, K. L. Pulvermacher, H. A. Olivares, J. Clardy, and J. Handelsman. 1998. Effect of canavanine from alfalfa seeds on the population biology of *Bacillus cereus*. Appl. Environ. Microbiol. 64:4683–4688.
14. Escudero, M. E., L. Velázquez, M. S. Di Genaro, and A. M. S. de Guzmán. 1999. Effectiveness of various disinfectants in the elimination of *Yersinia enterocolitica* on fresh lettuce. J. Food Prot. 62: 665–669.
15. Farkas, J., L. Mészáros, C. Mohácsi-Frakas, T. Sáray, and É. Andrássy. 1999. The role of ionizing radiation in minimal processing of precut vegetables with particular reference to the control of *Listeria monocytogenes*, p. 374–387. *In* F. A. R. Oliverira, J. C. Oliveira, M. E. Hendrickx, D. Korr, and L. G. M. Gorris (ed.), Processing foods: optimization and process assessment. CRC Press, New York.
16. Farkas, J., T. Sáray, C. Mohácsi-Frakas, K. Horti, and É. Andrássy. 1997. Effects of low-dose gamma radiation on shelf-life and microbiological safety of pre-cut/prepared vegetables. Adv. Food Sci. (CMTL) 19:111–119.
17. Garcia-Gimeno, R., and G. Zurera-Cosano. 1997. Determination of ready-to-eat vegetable shelf-life. J. Food Microbiol. 36:31–38.
18. Hagenmaier, R. D., and R. A. Baker. 1998. A survey of the microbial population and ethanol content of bagged salad. J. Food Prot. 61: 357–359.
19. Hayashi, T. 1998. Decontamination of dry food ingredients and seeds with "soft-electrons" (low energy electrons). Food Sci. Technol. Int. Tokyo 4:114–120.
20. Howard, L. R., G. H. Miller, and A. B. Wagner. 1995. Microbiological, chemical, and sensory changes in irradiated *pico de gallo*. J. Food Sci. 60:461–464.
21. Kader, A. A. 1986. Potential applications of ionizing radiation in postharvest handling of fresh fruits and vegetables. Food Technol. 40:117–121.
22. Kahan, R. S. 1969. Differential effects of small doses of ionizing radiation on the growth of onion seed and bulblets and on crop yields. Radiat. Bot. 9:175–178.
23. King, A. D., and H. R. Bolin. 1989. Physiological and microbiological storage stability of minimally processed fruits and vegetables. Food Technol. 43:132–135, 139.
24. King, A. D., J. A. Magnuson, T. Torok, and N. Goodman. 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. 56:459–461.
25. Lang, M. M., B. H. Ingham, and S. C. Ingham. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. Int. J. Food Microbiol. 58:73–82.
26. Mundt, J. O., and N. F. Hinkle. 1976. Bacteria within ovules and seeds. Appl. Environ. Microbiol. 32:694–698.
27. National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluation and recommendations on sprouted seeds. Int. J. Food Microbiol. 52:123–153.
28. Parakash, A., A. R. Guner, F. Caporaso, and D. M. Foley. 2000. Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food Sci. 65:549–553.
29. Rajkowski, K. T., and D. W. Thayer. 2000. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma radiation of inoculated sprouts. J. Food Prot. 63:871–875.
30. Ramakrishna, N., J. Lacey, and J. E. Smith. 1991. Effect of surface sterilization, fumigation and gamma irradiation on the microflora and germination of barley seeds. Int. J. Food Microbiol. 13:47–54.
31. Sattar, A. S. A., and M. A. Akhtar. 1990. Effect of irradiation and germination on trypsin inhibitor and protein content of chickpea (*Cicer arientinum*). Int. J. Vit. Nutr. Res. 60:402–406.
32. Sauer, D. B., and R. Burroughs. 1986. Disinfection of seed surface with sodium hypochlorite. Phytopathology 76:745–749.
33. Sumner, S. S., and D. L. Peters. 1997. Microbiology of vegetables, p. 87–114. *In* D. S. Smith, J. N. Cash, W.-K. Nip, and Y. H. Hui (ed.), Processing vegetables: science and technology. Technomic Publishing, Lancaster, Pa.
34. Thompson, S., and D. A. Powell. 2000. Risks associated with the consumption of fresh sprouts. Food Safety Network Technical Rep. 16. July 2000. Internet: http://www.plant.uoguelph.ca/safefood/micro-haz/sprouts-risk-sylvanus-jul00.htm.
35. U.S. Department of Health and Human Services. Irradiation in the production, processing and handling of food. Docket 99F-2673 B final rule. October 30, 2000. U.S. Department of Health and Human Services, Washington, D.C.
36. U.S. Department of Health and Human Services. Consumers advised of risks associated with raw sprouts. Press Release P99-13. July 9, 1999. U.S. Department of Health and Human Services, Washington, D.C.

37. Villavincio, A.L.C.H., J. Mancini-Filho, and H. Delincée. 1998. Application of different techniques to identify the effects of irradiation on Brazilian beans after six months storage. Radiat. Phys. Chem. 52: 161–166.
38. Weissinger, W. R., and L. R. Beuchat. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. J. Food Prot. 63:1475–1482.
39. Woodstock, L. W., and M. F. Combs. 1965. Effects of gamma-irradiation of corn seed on the respiration and growth of the seedling. Am. J. Bot. 52:563–569.